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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Patrick L. Iversen	EXAMINER: J. Epps
SERIAL NO.: 09/574,570	ART UNIT: 1635
FILED: May 17, 2000	CONFIRMATION NO. 7740
FOR: **ENYZME INHIBITORS FOR METABOLIC REDIRECTION**	

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Patrick L. Iversen, declare and affirm as follows:

I am the inventor of the above-referenced application.

I am currently Senior Vice President of Research and Development at AVI Biopharma Inc. I have been employed at AVI Biopharma, previously known as Antivirals Inc., since 1997.

I received a Ph.D. in the field of Biochemical Pharmacology and Toxicology from the University of Utah in 1984.

I have published numerous articles in the field of antisense technology and have worked with various types of oligonucleotide analogs in this area, including phosphorothioate-linked oligonucleotides and morpholino oligomers.

The following is a discussion of the effectiveness of uncharged, or substantially uncharged, antisense morpholino oligomers *in vivo*, which use is encompassed by pending claims in the above-referenced application.

A morpholino oligomer includes a sequence of morpholino subunits, which bear base-pairing moieties. These subunits are preferably connected by phosphorus-containing linkages which are largely uncharged. The combination of the morpholino subunits and the uncharged

phosphorus-containing linkages leads to favorable stacking of the base-pairing groups attached to the morpholino rings (see H. Kang *et al.*, 1992, enclosed). Various uncharged phosphorus-containing linkages, especially those encompassed by Figure 2B-B in the above-referenced application, can therefore be used. Most widely employed, however, are phosphorodiamidate-linked morpholino oligomers (PMOs).

The use of uncharged or substantially uncharged morpholino oligomers (MO) in antisense applications has been shown to overcome many of the drawbacks which are associated with charged antisense oligonucleotide analogs, such as the widely used phosphorothioate-linked oligonucleotide analogs.

Nonspecific effects observed with charged, RNAse-competent oligomers, such as the phosphorothioates, are generally attributed to nonspecific binding, both to nontargeted nucleic acids and to cellular proteins, and nonspecific RNAse activation. These effects are greatly reduced by the use of morpholino oligomers, in large part due to their minimal charge, or lack of charge, and mechanism of action, which is based on steric blocking rather than cleavage of the target. See, for example, the discussion in the enclosed review (P. Iversen, "Phosphoramidite Morpholino Oligomers", in *Antisense Drug Technology*, S.T. Crooke, ed., Marcel Dekker, Inc., New York, 2001), pp. 377-78. See also pp. S129, second column and following in Ricker *et al.*, 2002 (enclosed), and p. 254 of Kipshidze *et al.*, 2001 (enclosed).

The substantially uncharged MO's also enter more readily into cells *in vivo*, as evidenced by formation of MO-target RNA heteroduplexes *in vivo*; these heteroduplexes can be detected in body fluids following administration of the MO (PCT Pubn. Nos. WO 2000/45167; WO 2002/48405).

Furthermore, as noted in a review by Heasman ("Morpholino Oligos: Making Sense of Antisense?", *Dev. Biol.* **243**:219-14, 2002; enclosed), the target sites of morpholino oligomers (e.g. translation start codons, as well as splice sites in pre-mRNA) provide for more specific design of effective oligomers. (While the translation start site is most frequently targeted, successful targeting of pre-mRNA splice sites is described, e.g., in R.V. Giles *et al.*, *Antisense & Nucleic Acid Drug Dev.* **9**:213-220 (1999) and in P. Iversen, PCT Pubn. No. 2001/83740.)

In view of these advantages, many *in vivo* studies of antisense MO's have been carried out, both in studies of early gene development, in several different organisms (e.g. zebrafish, frog, sea urchin, chick, mouse; see Heasman review, cited above) and in various therapeutic models (Table 1). As Table 1 shows, *in vivo* antisense activity has been demonstrated by MOs directed to various targets (including p53, c-myc, TNF-α, cytochrome CYP3A2, β-HCG, matrix metalloproteinase-9, and integrin αV), and employing various routes of administration. As reported in the references cited, sequence-specific, dose-dependent inhibition of the targeted sequence by the PMO was observed in the majority of cases, while control (mismatched or scrambled) sequences generally gave little or no inhibition.

For example, as reported in Devi *et al.*, 2002b and Devi, 2002a (page 142) (all references enclosed), combination treatment with a PMO antisense to c-myc and to β-hCG inhibited tumor burden in mice by 60-75% compared to a scrambled-sequence control. In Ricker *et al*, 2002, cystic mice treated with a PMO antisense to c-myc had decreased relative kidney weight, improved renal function, and reduced cystic change compared with scrambled-sequence controls (Abstract; pages 127-128). In Qin *et al.*, 2000, antisense to TNF-α reduced levels of the protein by about 31%, compared to a negligible amount (about 1%) for a scrambled-sequence control. Arora *et al.*, 2000a shows that administration of a PMO antisense to c-myc reduced levels of the protein, and of PCNA (Fig. 3), significantly more than the same amount of a scrambled-sequence control (Fig. 1, lane 5 vs. lane 8; also reported in Arora *et al.* 2002). The latter also reports that levels of CYP3A2 enzyme were reduced to 20-25% of vehicle control by an antisense PMO, vs. about 75% of vehicle control for a scrambled-sequence control (Fig. 7; page 1014).

Kipshidze *et al.*, 2001 and 2002 and Kim *et al.*, 2001 describe *in vivo* effectiveness of antisense PMOs in inhibiting restenosis in rabbit and porcine models, administered by catheter or conjugated to a microbubble suspension. The latter systems are also described in PCT Publication No. WO 2000/02588. Effectiveness of antisense PMOs directed to cytochrome P450 enzymes *in vivo*, administered orally or transdermally, is described in PCT Publication No. WO 2001/87286; Arora *et al.* 2002 also describes oral administration.

Table 1. Reports of Morpholino Antisense Activity *in vivo* in Therapeutic Animal Models

Target	Route	Species	Model
p53	IP	Rat	Liver regeneration
c-myc	IP	Mouse	Polycystic kidney disease
c-myc	IP	Mouse	Cancer
c-myc	IP	Rat	Liver regeneration
c-myc	Endoluminal catheter	Rabbit	Restenosis
c-myc	Endoluminal catheter	Pig	Restenosis
c-myc	IV microbubbles	Pig	Restenosis
TNF-α	Pulmonary inhalation	Mouse	Pulmonary inflammation
TNF-α	IV	Rat	Septic shock
CYP3A2	IP; IV; Oral; Transdermal	Rat	Drug metabolism
β-HCG; c-myc	IP	Mouse	Prostate cancer
Integrin αV	Intratumoral	Mouse	Glioblastoma
MMP-9	Local or IP	Mouse	Angiogenesis

[a] See paragraph bridging pages 142-143.

As shown by the studies described above and in the enclosed references, morpholino oligomers having uncharged, phosphorus-based linkages have shown sequence-specific antisense activity *in vivo* in a variety of animal models and targets.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Oct. 24, 2002
Date

Patrick L. Iversen